Supporting Information

Synthesis and Biological Evaluation of Pyrrolidine Functionalized Nucleoside Analogs

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Instrumentation.

NMR characterization. NMR spectra were acquired with a Varian Inova 400 MHz spectrometer (Varian Inc) or Bruker Advance 400 MHz instrument (Bruker BioSpin) using DMSO-d_6 (Cambridge Isotope Laboratories, Inc) or D_2O (Cambridge Isotope Laboratories, Inc) as a solvent.

HPLC purification. HPLC purification of compounds synthesized in this study was carried out with an Agilent Technologies model 1100 HPLC system equipped with a photodiode array UV detector. Unless specified otherwise, UV absorbance was monitored at 254 nm.

HPLC System 1. HPLC purification of compounds **1-17** was carried out using a semi-preparative Zorbax Eclipse XDB-C18 column (9.4 mm x 250 mm, 5 μm, Agilent Technologies, Inc., eluted with a linear gradient of acetonitrile (B) in water (A) at a flow rate of 3 mL/min.

HPLC System 2. Compounds **8** and **9** monophosphates (**8-MP** and **9-MP**) were purified by reversed phase semi-preparative HPLC using a Synergi 4u Hydro-RP 80A column (10 mm x 250 mm, Phenomenex Inc) eluted at a flow rate of 3 mL/min and maintained at 25ºC using 150 mM ammonium acetate (A) and MeOH (B). Solvent composition was held at 0% B was used for the first two minutes of each run followed by a linear gradient of 0% to 7% B in 10 min and an increase to 67% B over the next 20 min. The column was maintained at 67% B over the next three min., after which the gradient was increased linearly to 77% B over the next 15 min., followed by column equilibration at 0% B for 15 min.

Tandem mass spectrometry characterization. All synthetic compounds were characterized by MS, MS^2 and MS^3 using an Agilent MSD SL ion trap mass spectrometer (Agilent Technologies, Inc). The instrument was operated in the ESI^+ mode. Target ion abundance value was set to 30,000, the maximum accumulation time was 300 milliseconds, and 6 scans were taken per average. A typical fragmentation amplitude was 0.7 V, with a scan width of 1.2 *m/z*. Nitrogen was used as a nebulizing (15 psi) and a drying gas (5 L/min, 200 °C). Electrospray ionization was achieved at a spray voltage of 3-3.5 kV. Samples were dissolved in a 1:1 mixture of ACN and 0.1% acetic acid and infused at a flow rate of 10-15 μL/min using a syringe pump. The mass spectrometer was operated in a full scan mode over the range of *m/z* 15-600.

Accurate mass measurements. High resolution mass spectra were obtained using a Bruker BioTOF II (Bruker Corp.), a reflectron electrospray ionization-time of flight instrument operated in the ESI^+ mode. HPLC purified nucleoside analogs were dissolved in MeOH and infused using a syringe pump. Poly(ethyleneglycol) (average molar mass = 200) was used as the internal calibrant. Data processing was done by using Bruker Daltonics software.

Table S1. Accurate mass data for compounds **1-17** obtained by ESI-TOF analysis.

compound	formula M+H	m/z calc	m/z obs	Error/ppm
1a	C14H20N5O5	338.1464	338.14630	-1.18
1b	C14H20N5O5	338.1464	338.14571	0.56
1c	C14H20N5O5	338.1464	338.14556	1.01
2a	C14H20N5O4	322.1515	322.15070	0.87
2b	C14H20N5O4	322.1515	322.15068	0.93
3	C14H19FN5O3	324.1472	324.14860	-4.34
5	C14H21N6O5	353.1573	353.15670	1.82
6	C13H20N3O6	314.1352	314.12780	23.59
7	C14H22N3O6	328.1509	328.14860	6.89
8	C14H22N3O4	296.1610	296.16010	3.15
9	C13H20N3O4	282.1454	282.14550	-0.42
10	C14H20N5O4	322.1515	322.14730	13.13
11	C13H20N3O5	298.1403	298.13960	2.34
12	C14H22N3O5	312.1559	312.15560	0.96
13	C14H22N3O3	280.1661	280.16360	8.92
15	C8H12N3O	166.0980	166.09870	-4.21
16	C9H15N4O	195.1246	195.12365	2.00
17	C12H17F2N4	255.1421	255.14230	-0.78

Table S2. Anti-HSV testing results for pyrrolidine substituted nucleosides prepared in this study.

Compound	% Cell viability	
	50 μM	**100 μM**
1a	105.7±29.0	77.3±10.9
1b	131.4±17.6	95.8±15.3
1c	143.8±16.0	80.5±8.5
2a	106.2±21.6	73.6±4.1
2b	119.2±16.6	80.4±10.0
3	124.7±21.8	93.9±7.3
5	159.0±17.0	124.1±17.7
6	126.9±2.9	97.9±8.0
10	152.6±19.2	120.5±12.0
Control	100±14.1	100±14.1

Table S3. Percentages of viable DU145 cancer cells following treatment with analogs **1-10**.

Compound	% Cell viability			
	50 μM		100 μM	
	Trial 1	Trail 2	Trial 1	Trail 2
1a	105.7±29.0	116.2±9.7	77.3±10.9	99.7±11.0
1b	131.4±17.6	114.0±14.7	95.8±15.3	77.8±13.3
1c	143.8±16.0	107.3±7.3	80.5±8.5	83.6±9.2
2a	106.2±21.6	97.4±5.0	73.6±4.1	73.9±11.1
2b	119.2±16.6	95.0±5.3	80.4±10.0	74.5±2.1
3	124.7±21.8	96.2±21.2	93.9±7.3	67.8±3.3
5	159.0±17.0	108.5±9.0	124.1±17.7	92.4±8.1
6	126.9±2.9	124.0±7.7	97.9±8.0	112.8±14.0
10	152.6±19.2	111.4±9.0	120.5±12.0	85.1±12.5
11			92.9 ± 13.0	
13			83.8 ± 9.2	
15			95.5 ± 11.7	
Control	100±14.1	100±16.5	100±14.1	100±16.5

Table S4. Percentages of viable CCRF-CEM cancer cells following treatment with nucleoside analogs.

Compound	Concentration		
	1 µM	**10 µM**	**100 µM**
1a	112.0 ± 9.7	106.5 ± 26.0	94.7 ± 12.8
6	104.5 ± 21.4	106.4 ± 18.8	105.8 ± 6.3
11	103.9 ± 11.8	106.5 ± 23.3	105.9 ± 9.1
13	107.0 ± 5.3	103.6 ± 16.1	90.3 ± 18.2
15	116.5 ± 8.9	109.0 ± 9.5	99.9 ± 13.7

Table S5. Percentages of viable HL-60 cancer cells following treatment with nucleoside analogs.

Compound	Concentration		
	1 μM	**10 μM**	**100 μM**
1a	105.3 ± 28.4	105.0 ± 21.3	99.3 ± 29.0
6	106.5 ± 22.8	102.6 ± 40.2	98.0 ± 27.6
11	92.9 ± 18.5	102.4 ± 23.2	98.2 ± 29.1
13	100.1 ± 14.0	97.4 ± 17.7	93.6 ± 10.9
15	107.6 ± 29.3	108.8 ± 24.3	105.7 ± 27.0

Figure S1. NOESY spectrum of compound **9**.